

Abstract

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Diploma thesis title: Study of deproteinization of milk samples via salting-out induced phase separation and automation by Lab-In-Syringe

A novel approach to sample deproteinization based on homogeneous liquid-liquid extraction with salting-out induced phase separation was explored. A methodology was developed, experimentally tested, and optimized that allows automated centrifuge-less deproteinization of milk samples using the flow-batch technique Lab-In-Syringe.

As in other sample preparation methods, the aim was to eliminate the sample matrix and obtain an organic phase that could be submitted to analysis. The thesis includes a range of preliminary experiments performed offline, i.e. without the intended instrumentation, as well as optimization experiments performed online using a Lab-In-Syringe system. Salting out agents and three solvents were tested as well as phase ratios and operational parameters on the Lab-In-Syringe system, including system configuration. In the method optimized by the work reported in this thesis, milk proteins were found effectively denatured and precipitated by the water-miscible solvent acetonitrile, which also serves as possible extraction solvent for analytes of interest in the milk sample. Phase separation was induced by the addition of a mixed solution of MgSO_4 and NaCl as salting-out agents.

The automation was based on the Lab-In-Syringe technique with in-syringe magnetic stirring, providing thorough and homogenous mixing inside the syringe void upon the aspiration of the sample and individual liquid reagents. For automated centrifuge-less deproteinization and phase separation, the following procedure was found optimal: diluted milk sample treated by acetonitrile and subsequently by the previously mentioned salting-out solution. Moreover, cleaning protocols were critically examined. In the extracts obtained from the optimized protocol, no traces of proteins were found. All experiments were documented by photographs.

The developed system and method will be coupled online to HPLC in a future work to which this thesis was aimed to contribute the first part, system setup and method development.